Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
29 MAR 2011	**research**	**01-01-2010 to 01-01-2011**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Specific recognition and detection of MRSA based on molecular probes comprised of lytic phage and antibody	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Auburn University,College of Veterinary Medicine,Auburn,AL,36849**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) **81st Medical Group, Clinical Research Laboratory, 301 Fisher Street, Keesler AFB, MS, 39534**	10. SPONSOR/MONITOR'S ACRONYM(S) **81 MDG, CRL**
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
A Q-sense E4 QCM system was employed to study bacteria-phage interactions.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**1**	**1**	

Specific recognition and detection of MRSA based on molecular probes comprised of lytic phage and antibody

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Methicillin resistant strains of *Staphylococcus aureus* (MRSA) are implicated in serious infections and nosocomial outbreaks. MRSA show resistance to a wide range of antibiotics thus limiting the treatment options. Therefore, rapid detection of MRSA is of key importance in prevention and diagnosis of infections caused by antibiotic resistant *S. aureus*. Currently existing methods of MRSA detection have some limitations and lack sensitivity or specificity. We examined a new approach in specific recognition and detection of MRSA, including identification of bacteria together with conformation of MRSA in real time. For this purpose we use a newly isolated *S.aureus* bacteriophage with a wide spectrum of hosts (including MRSA strains) together with monoclonal antibody against a penicillin-binding protein (PBP 2a). PBP 2a is a cell wall protein and it is responsible for antibiotic resistivity of MRSA. We showed that simultaneous recognition of Staphylococcus bacteria and PBP 2a protein increases specificity and reliability of MRSA detection.

A Q-sense E4 QCM-D system (Sweden) was employed to study bacteria-phage interactions. Lytic phages were constructed into hollow spherical particles upon exposure to a chloroform-water interface. These particles were converted into monolayers and deposited onto QCM-D crystals using Langmuir-Blodgett technique [1]. Biding of MRSA has been manifested by frequency and dissipation changes (Fig.1). Similar results were obtained with antibiotic sensitive *S. aureus*.

An agglutination test was carried out using a latex reagent sensitized with antibody against PBP 2a (Denka Seiken Co., Ltd, Tokyo, Japan). The bacterial cells were mixed with the latex reagent on a test card. A visible agglutination occurred in about three minutes for MRSA. For the antibiotic sensitive *S. aureus* strains no agglutination occurred, even after one hour.

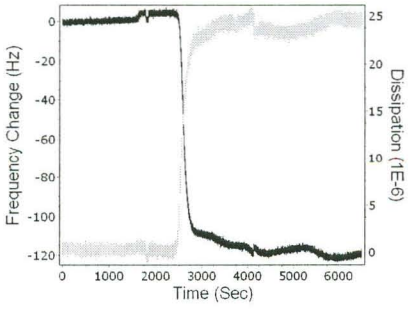

Figure 1. Time change in frequency and dissipation energy during MRSA binding

The interaction between PBP 2a protein and antibodies is not specific for *Staphylococcus aureus* because other bacteria have antibiotic binding proteins with large sequence similarity to PBP 2a. Therefore in our work we used *S.aureus* bacteriophage with wide spectrum of hosts together with antibodies against PBP 2a protein. In order to build a biosensor to specifically detect and identify MRSA we will employ a device with two parallel channels. One channel will have a *S.aureus* bacteriophage monolayer as a sensor probe, while the sensor of another channel will be covered with PBP 2a specific antibodies. Consequently, one channel will identify *S.aureus* bacteria, while another one will be sensitive to the antibiotic-binding protein. When signals coming from two channels are positive it would indicate the specific detection of MRSA.

Acknowledgements Supported by grants from USAF CRADA 07-277-60MDG-01 and Auburn University AUDFS. The views expressed in this article are those of the authors, and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

[1] Guntupalli, R, Sorokulova, I, Long, R, Olsen, E, Neely, W, Vodyanoy, V (2010) Phage Langmuir monolayers and Langmuir-Blodgett films. Colloids and Surfaces B-Biointerfaces 82: 182-189.